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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Our File:	10875.77
Applicant:	Desgroseillers *et al.*
Serial No.:	09/316,048
Filed:	05/21/99
Group Art Unit :	1632
Examiner:	Ram R. Shukla
Title:	MAMMALIAN STAUFEN AND USE THEREOF

AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 22202
U.S.A.

Dear Sir:

In response to the Final Official Action mailed on September 10, 2002, in connection with the above-identified patent application, and in accordance with Rule 115 of the Rules of Practice, please consider the following amendments and remarks. Response to the Final Office Action is due on March 10, 2003 with a three-month (3) extension of time. The Applicants submit concurrently a Petition for Extension of Time for two months, to and including March 10, 2003, accompanied by the required fee. Please also find enclosed a Request for Continued Examination, accompanied by the required fee, a Sequence Listing and its accompanying diskette as well as the statement under 37 CFR 1.825(a) and (b).

IN THE DISCLOSURE:

Kindly replace page 12, line 5 to page 13, line 2 by the following:

"Figure 1A shows an alignment of the two cDNAs of the human *staufen* cDNAs, designated T1 (SEQ ID NO: 1) and T2 (SEQ ID NO: 3) and the translation of the putative protein sequences thereof, starting at amino acid no. –81 and 1, respectively, and presented as the amino acid sequences in SEQ ID NO: 2

and SEQ ID NO: 4, respectively. The numbers refer to the sequence of the short cDNA. The positions of the 4 dsRNA-binding consensus domains (RBD1 to RBD4) and of the tubulin-binding domain (TBD) are indicated between brackets above the sequence. The sequences were deposited in the GenBank database under accession numbers AF061938 and AF061939.

Figure 1B is similar to Figure 1 but shows the alternative splicing which occurs in the human *staufen* gene and gives rise to 4 alternatively spliced transcripts, namely T1, a 3142 bp nucleotide sequence appearing in SEQ ID NO: 5 and encoding from nucleotide 288 to 1775 the protein appearing in SEQ ID NO: 4; T2, a 3217 bp nucleotide sequence discussed above and appearing in SEQ ID NO: 3 and also encoding from nucleotide 363 to 1850 the protein appearing in SEQ ID NO: 4; T3 (designated T1 in Fig 1A), a 3506 bp nucleotide sequence appearing in SEQ ID NO: 1 and encoding from nucleotide 409 to 2139 the protein appearing in SEQ ID NO: 2; and T4, a 3348 bp nucleotide sequence appearing in SEQ ID NO: 6 and encoding from nucleotide 494 to 1981 the protein appearing in SEQ ID NO: 4. These 4 transcripts therefore give rise to the two proteins as described in Figure 1 and in the text below..

Figure 1C shows the nucleic acid (SEQ ID NO: 7) and predicted amino acid sequence (SEQ ID NO: 8) of mouse *staufen* .

Figure 1D shows an alignment of the mouse (SEQ ID NO: 8) and human Staufen (SEQ ID NO: 4), highlighting the significant conservation of the protein during evolution. As per Figure 1, the 4 dsRNA binding domains (RBD) and tubulin-binding domains are highlighted.

Figure 1' shows an alignment between phylogenetically different Staufen proteins of *Drosophila* (SEQ ID NO: 9), *C. elegans* (SEQ ID NO: 10) and human (SEQ ID NO: 4). This alignment permits the elaboration of a consensus sequence for *staufen*. As shown in Figure 1B, T1, T2 and T4 give rise to the short protein of 55 kDa (SEQ ID NO: 4) while T3 gives rise to the 63 kDa protein (SEQ ID NO: 2). Figure 1'B shows an alignment between a region comprising the human Staufen tubulin-binding domain (SEQ ID NO: 11) and the human MAP1B microtubule-binding domain (SEQ ID NO: 12)."

At pages 42, line 4 to p. 43, line 7, kindly replace by the following:

"annealed complementary oligonucleotides 5'-AGCTTAATTAGCTGAC-3' (SEQ ID NO:13) and 5'-AGCTGTCAGCTAATTA-3' (SEQ ID NO:14). The MBP/mSTAU fusion protein, containing the full-length mStau sequence, was generated by PCR amplification with Vent DNA polymerase (New England BioLabs), using the primer pair 5'-CCTGGATCCGAAAG-TATAGCTTCTACCATTG-3' (SEQ ID NO:15) and 5'-TACATAAGCTTCTAGAT-GGCCAGAAAAGGTTCAGCA-3' (SEQ ID NO:16). The resulting 1562 bp fragment was digested with HindIII and BamHI, and ligated in the pMal-c vector. The C-terminal fragment (mSTAU-C) was amplified with the primer pair 5'-GGATGAATCCTATTAGTAGACTTGCAC-3' (SEQ ID NO:17) and 5'-TACATAAGC-TTCTAGATGGCCAGAAAAGGTTCAG-CA-3' (SEQ ID NO:23), digested with HindIII and cloned in the EagI* and HndIII sites of pMal-c. EagI* was created by filling in the cohesive ends of EagI-digested pMal-c vector using the Klenow fragment of DNA polymerase I. This fusion vector was then digested with SacI and EcoRI and the resulting fragment was subcloned in the pMal-stop vector to generate the mSTAU-RBD3 construct. The mSTAU-TBD construct was prepared by PCR using the primer pair 5'-GCTCTAGATTCAAAG-TTCCCCAGGC-GCAG-3' (SEQ ID NO:18) and 5'-TTTAAGCTTCTCAGA-GGGTCTAGT-GCGAG-3' (SEQ ID NO:19); the product was digested with XbaI and HindIII and cloned in the pMal-stop vector. mSTAU-RBD2 and mSTAU-RBD1 were constructed by first amplifying a fragment using the primer pair 5'-CAATGTATAAGCCCGTGGACCC-3' (SEQ ID NO:20) and 5'-AAAAAGCTTGTGCAAGTCTACTAATAGGATTCACC-3' (SEQ ID NO:21). The resulting product was digested with HindIII and cloned in the EagI* and HindIII sites of the pMal-stop vector. This vector was then used to purify the 398 bp PstI and HindIII fragment, which was subcloned in the pMAL-stop vector to generate the mSTAU-RBD2 construct. In the same way, the mSTAU-RBD1 vector was obtained by digestion with SmaI and StuI, followed by recircularization of the digestion product using T4 DNA ligase. The mSTAU-RBD4 was PCR amplified using the primer pair 5'-ATAGCCCGAGAGTTGTTG-3' (SEQ ID NO:22) and 5'-TACAT-AAGCTTCTAGATGGC-CAGAAAAGGTTCAGCA-3' (SEQ ID NO:23)."

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